Supplemental Material

An analysis of cumulative risks based on biomonitoring data for six phthalates using the Maximum Cumulative Ratio

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Attached Files

1. "Reyes_EHP_Phthalates_US_metabolites.csv" contains information about the National Health and Nutrition Examination Survey (NHANES) metabolite code names along with Tolerable Daily Intake (TDI), molecular weight of the parent phthalate, molecular weight of the metabolite, and names and abbreviations of the corresponding metabolites and phthalates.
2. "Reyes_EHP_Phthalates_US_MCR.csv" contains the following information:
    - Data on the surveyed individuals from the original NHANES data files that includes
        - Identifying information for individual;
        - Demographic information used in defining the creatinine levels and in investigating sub populations based on age, gender, ethnicity;
        - Concentration of phthalate metabolites and creatinine in urine.
    - Calculated values for each individual;
        - Daily intake of each of the six phthalate;
        - Hazard Quotients associated with each phthalate's daily intake;
        - Values of Hazard Index and the Maximum Cumulative Ratio.
3. "Reyes_EHP_Phthalates_US_MCR_definitions.csv" and the definitions and units of the fields in "Reyes_EHP_Phthalates_US_MCR.csv".

Description of statistical tests used

Significance tests were performed with the Hazard Index (HI) exploring the differences in the means of HI by 1) Age (6 – 17, 18+), 2) Gender (Female, Male), and 3) Ethnicity (Mexican American, Hispanic, Non-Hispanic White, Non-Hispanic Black, and Other). The tests performed were A) a t-test (and Tukey test) exploring the differences in the means as well as B) a proportion test (and χ^2 test) looking at differences in the proportion of participants with $HI > 1$. Among the demographic categories with only two options (i.e. Age and Gender), the difference tests were a t-test (non-paired and assuming unequal variance) and a proportions test. Among the demographic categories with more than two options (i.e. Ethnicity), the differences tests were a χ^2 test and a Tukey test.

Phthalate	Molecular weight of parent MW_p (g/mol)	Metabolite	Molecular weight of metabolite MW_m (g/mol)	Fractional urinary excretion rates (F_{UE}) (%)
di-n-butyl phthalate (DBP)	278.34	monobutyl phthalate (MBP)	222.24	84
diisobutyl phthalate (DIBP)	278.34	monoisobutyl phthalate (MIBP)	222.24	70.3
butyl benzyl phthalate (BBP)	312.36	monobenzyl phthalate (MBZP)	256.25	73
di(2-ethylhexyl) phthalate (DEHP)	390.56	mono(2-ethyl-5-carboxypentyl) phthalate (MECPP)	308.33	13.2
		mono(2-ethyl-5-oxohexyl) phthalate (MEOHP)	292.33	10.9
		mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP)	294.35	14.9
		mono(2-ethylhexyl) phthalate (MEHP)	278.34	6.2
diisononyl phthalate (DINP)	418.61	monoisononyl phthalate (MINP)	292.37	3
		mono(carboxyoctyl) phthalate (MCOP)	322.36	9.9
diisodecyl phthalate (DIDP)	446.68	mono(carboxynonyl) phthalate (MCNP)	336.38	9.9

Table S1. Molecular weights and fractional urinary excretion rates of phthalates and corresponding metabolites from the 2013-2014 NHANES cycle.

HI vs MCR	Category	Slope	Intercept	Adjusted r^2	Pearson's r	Spearman's r
Overall	---	-0.395	-0.421	0.261	-0.511	-0.411
HQ_M	DEHP	-0.316	-0.295	0.225	-0.475	-0.355
	DBP	-0.205	-0.222	0.070	-0.265	-0.178
	DINP	-0.541	-0.578	0.385	-0.620	-0.586
	DIDP	**-0.282**	**-0.238**	0.123	**-0.351**	-0.331
Age	6 - 17	-0.434	-0.480	0.300	-0.548	-0.453
	18+	-0.345	-0.326	0.210	-0.458	-0.348
Gender	Female	-0.388	-0.418	0.251	-0.501	-0.408
	Male	-0.402	-0.424	0.273	-0.522	-0.413
Ethnicity	White	-0.416	-0.434	0.303	-0.550	-0.446
	Other	-0.407	-0.485	0.272	-0.522	-0.464
	Black	-0.366	-0.393	0.215	-0.464	-0.364
	Mexican American	-0.374	-0.374	0.219	-0.468	-0.351
	Hispanic	-0.370	-0.396	0.265	-0.515	-0.407

Table S2. Linear regression parameters presented by HQ_M, age, gender, and ethnicity. All regression parameters are highly statistically significant ($p < 0.001$) except the slope of DIDP ($p = 0.079$) and the intercept of DIDP ($p = 0.042$). **Bolded** = not statistically significant at $p < 0.001$.

Hazard Index comparison	Difference Test	Category	p value (with 95% CI)
$HI > 1$	proportions	Age	0.44
	proportions	Gender	1.00
	χ^2	Race	0.41
mean HI difference	t-test	Age	<0.001
	t-test	Gender	0.93
	Tukey test	Race	ranges 0.31-1.00

Table S3: Results of the statistical difference tests of the Hazard Index.

	I	II	IIIA	IIIB	Total
BBP	0/0/0	0/0/0	0/0/0	0/0/0	0/0/0
DBP	0/0/0	958/885/990	0/0/0	1/1/1	959/886/991
DEHP	3/3/3	712/657/698	1/1/1	1/0/1	717/661/703
DIBP	0/0/0	0/0/0	0/0/0	0/0/0	0/0/0
DIDP	1/0/1	25/0/13	0/0/0	0/0/0	26/0/14
DINP	8/9/8	947/1096/943	5/10/3	1/1/1	961/1116/995
Total	12/12/12	2642/2638/2644	6/11/4	3/2/3	2663

Table S4. Summary of phthalates that produced the HQ_M by method and group. A count of phthalates that produced the HQ_M for all individuals in the 2013-2014 cycle of NHANES by phthalate and Group for (A) this work, (B) using the metabolites from [ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1016/j.yrtph.2014.04.019", "ISSN" : "10960295", "PMID" : "24815596", "abstract" : "Exposures to multiple chemicals may contribute to increased risk of similar adverse effects. Cumulative risk may be estimated using a hazard index (HI), the sum of individual hazard quotients (HQ, ratio of exposure to the reference value). We demonstrate the HI approach for five phthalates: di(2-ethylhexyl) phthalate (DEHP), di-n-butyl phthalate (DBP), diisobutyl phthalate (DiBP), diisononyl phthalate (DiNP), and butyl benzyl phthalate (BBP). Phthalate exposure for the US general population is estimated using urine metabolite levels from NHANES, extrapolating to ingested 'dose' using the creatinine correction approach. We used two sets of reference values: European Union Tolerable Daily Intakes and Denmark Environmental Protection Agency Derived No Effect Levels. We also investigated the use of an alternate reference value for DEHP, derived from a recent study on male reproductive system development. HQs and HIs were calculated for the total population ages 6. years and older, as well as for men and women of approximate reproductive age (18-39. years), and children (6-11. years). Median HQs ranged from <0.01 for BBP, to ~0.1 (using established values) or ~2 (using an alternate value) for DEHP. Median HIs were <0.30 (95th percentiles just > 1.0), and were driven by DEHP and DBP exposures. ?? 2014.", "author" : [{ "dropping-particle" : "", "family" : "Christensen", "given" : "Krista L Y", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Makris", "given" : "Susan L.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Lorber", "given" : "Matthew", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Regulatory Toxicology and Pharmacology", "id" : "ITEM-1", "issue" : "3", "issued" : { "date-parts" : [["2014"]] }, "page" : "380-389", "publisher" : "Elsevier Inc.", "title" : "Generation of hazard indices for cumulative exposure to phthalates for use in cumulative risk assessment", "type" : "article-journal", "volume" : "69" }, "uris" : ["http://www.mendeley.com/documents/?uuid=1e16b722-485c-44b3-954e-d55f380fd00b"] }], "mendeley" : { "formattedCitation" : "(Christensen et al. 2014)", "plainTextFormattedCitation" : "(Christensen et al. 2014)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }], (C) setting the limit of detection to zero. The numbers in each cell read (A) / (B) / (C).

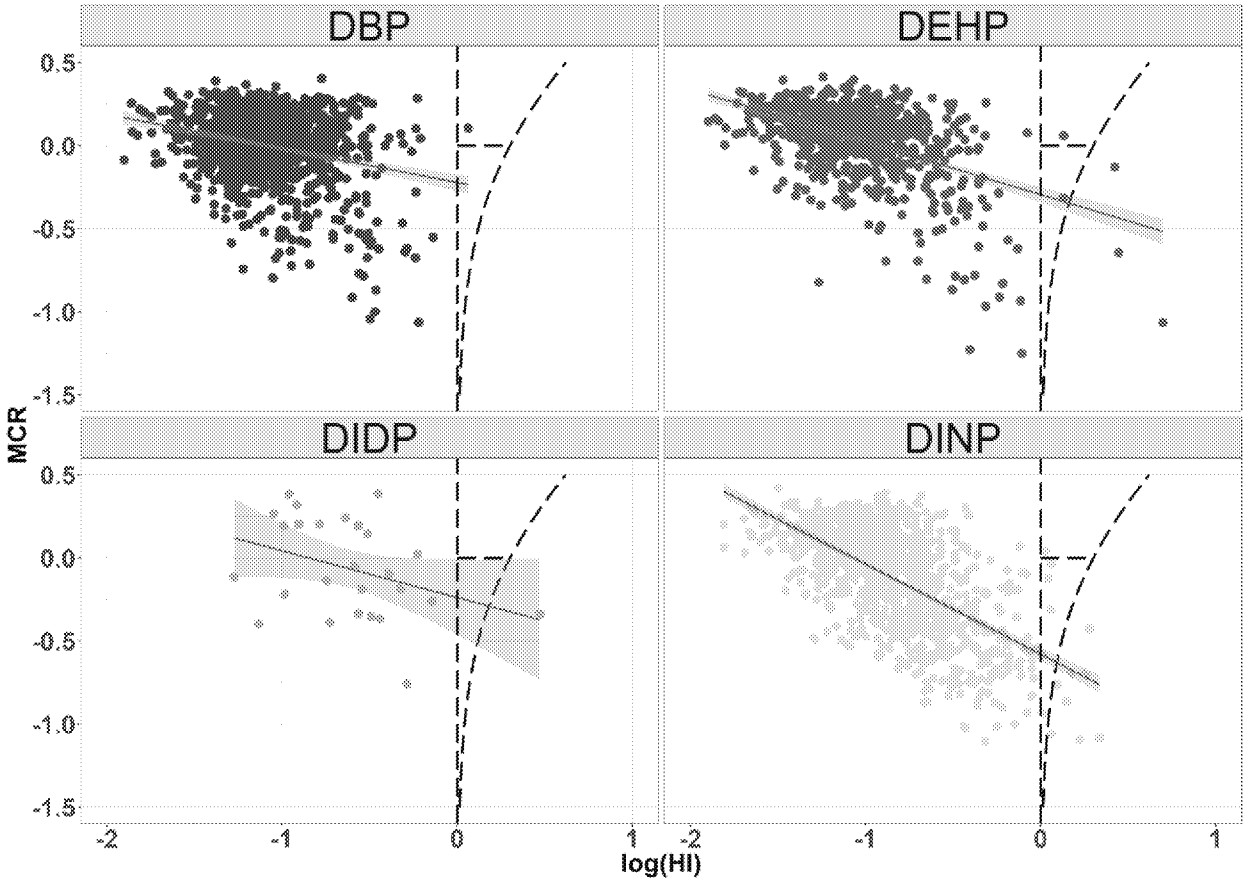

Figure S1. HI versus MCR by HQ_M. log Hazard index versus $\log(MCR - 1)$ of six phthalates for 2,663 individuals from the 2013-2014 NHANES cycle identified by the phthalates that produced the HQ_M separated by Groups I, II, IIIA, and IIIB.

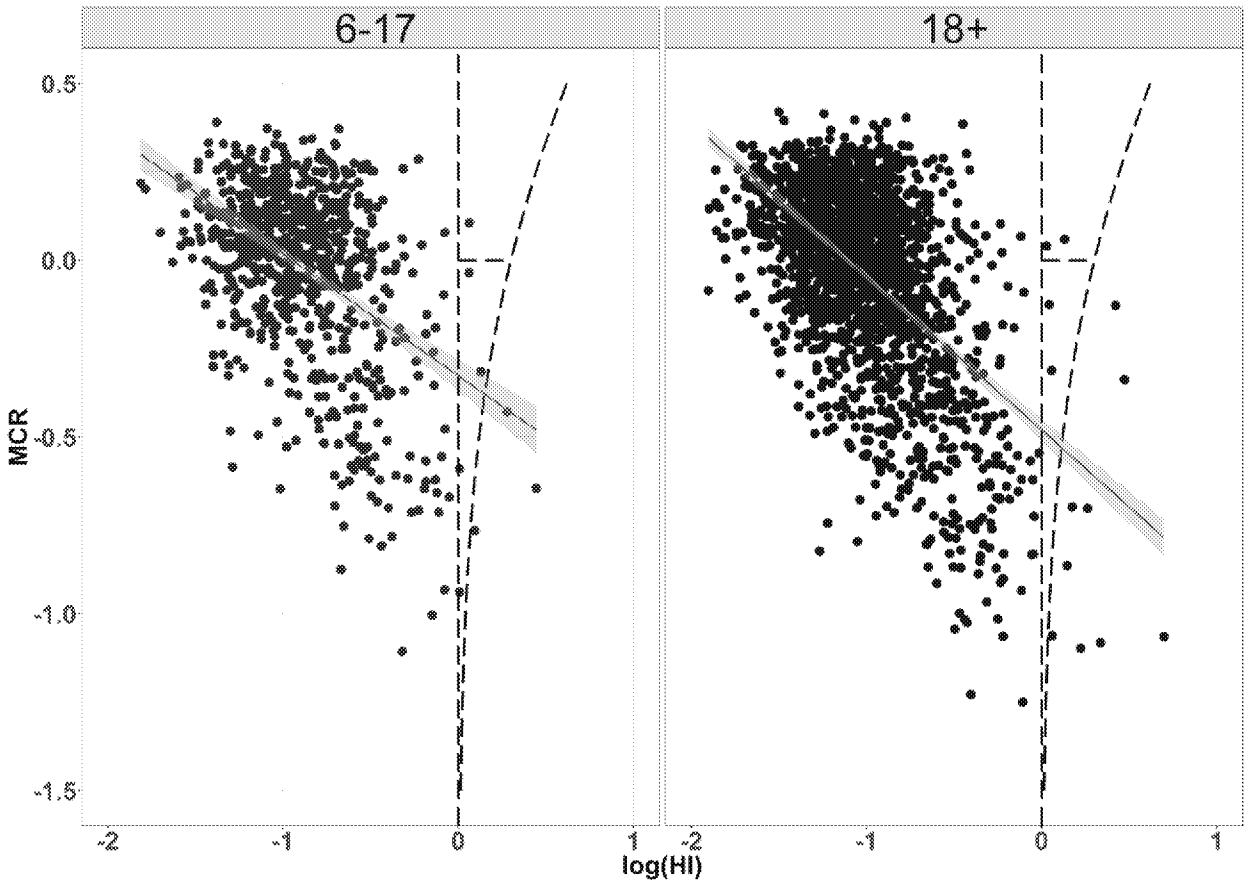

Figure S2. HI versus MCR by age. log Hazard index versus $\log(MCR - 1)$ of six phthalates for 2,663 individuals from the 2013-2014 NHANES cycle identified by age group separated by Groups I, II, IIIA, and IIIB.

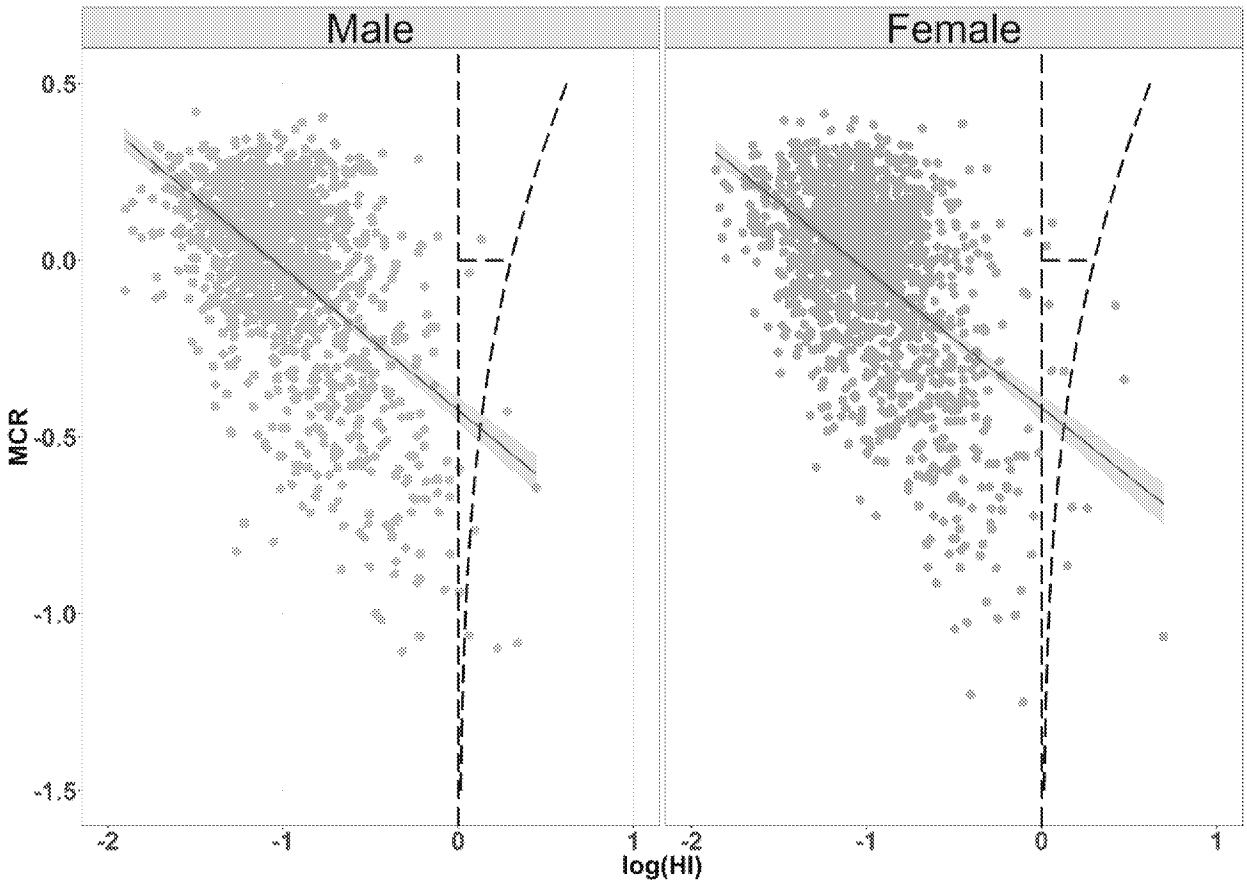

Figure S3. HI versus MCR by gender. log Hazard index versus $\log(MCR - 1)$ of six phthalates for 2,663 individuals from the 2013-2014 NHANES cycle identified by gender separated by Groups I, II, IIIA, and IIIB.

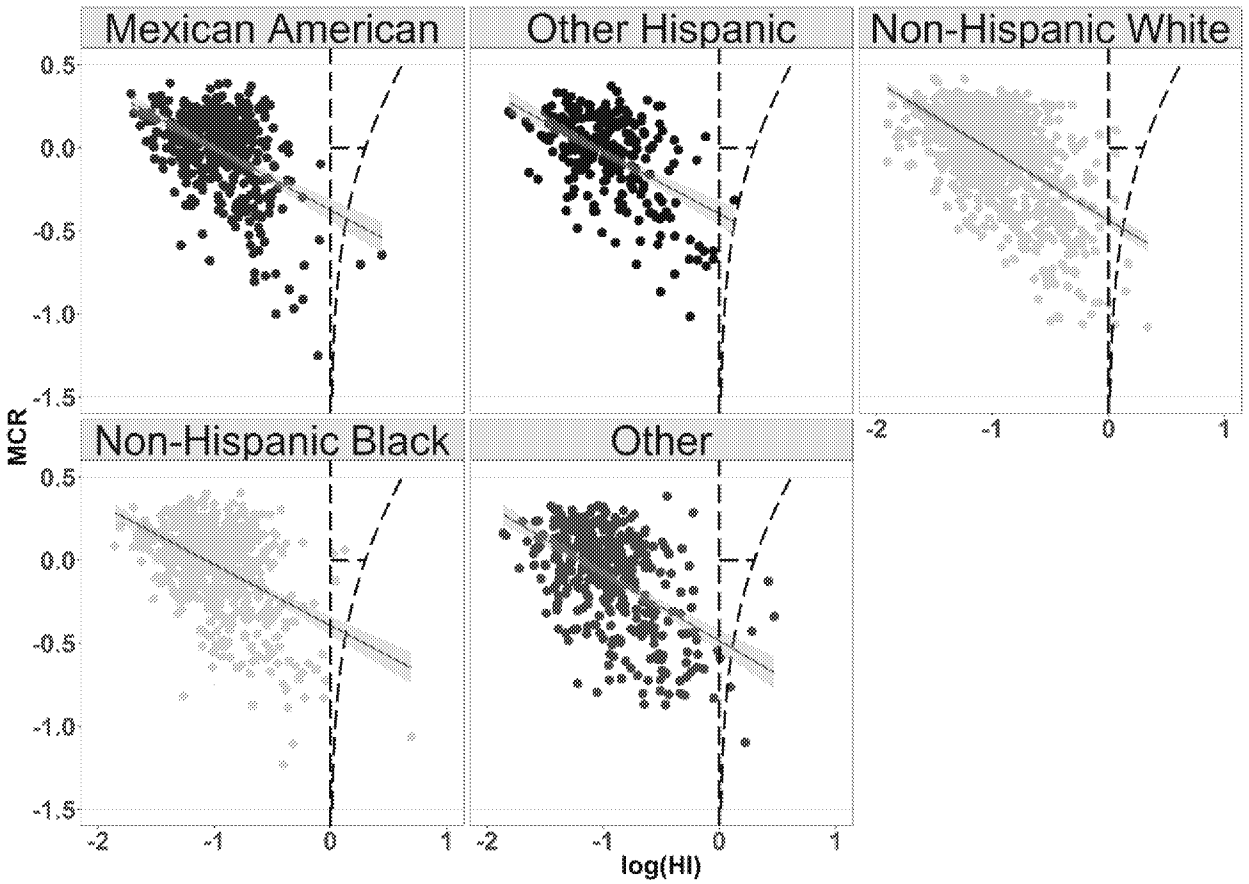

Figure S4. HI versus MCR by ethnicity. log Hazard index versus $\log(MCR - 1)$ of six phthalates for 2,663 individuals from the 2013-2014 NHANES cycle identified by ethnicity separated by Groups I, II, IIIA, and IIIB.

References

[ADDIN Mendeley Bibliography CSL_BIBLIOGRAPHY]